Original Research Article

IN-VITRO ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF MANNOSE/GLUCOSE-BINDING *PTEROCARPUS OSUN* CRAIB. SEEDS LECTIN

ABSTRACT

Objective: This study was carried out to purify and characterize a carbohydrate-binding and cell-agglutinating protein, lectin, from *Pterocarpus osun* seeds and also to evaluate its antioxidant and antibacterial potential.

Methods: Isolation and purification of the lectin were done by ammonium sulphate precipitation and size exclusion chromatography on Sephadex G-100. Physicochemical properties of the lectin were determined and antioxidant activity was evaluated by DPPH radical scavenging, lipid peroxidation inhibition and ferric reducing antioxidant potential assay. Disc diffusion methods was used for antibacterial effect.

Results: Lectin was detected in the seeds and was able to agglutinate native and enzyme-treated rabbit erythrocytes but only enzyme-treated erythrocytes of human blood were agglutinated. Mannose, Maltose and α-methylmannoside inhibited the divalent cation independent hemagglutinating activity, which was stable up to 60°C and at pH range of 3-13. It showed antioxidant activity with IC_{50} of 1.17 ± 0.08, 0.58 ± 0.03, and 2.51 ± 0.03 mg/ml for those methods respectively. No antibacterial activity was observed.

Conclusion: *Pterocarpus osun* seeds lectin possess properties similar to other lectins from *Dalbergieae* tribe and its ability to scavenge free radical and inhibit lipid peroxidation show the presence of a valuable health promoting agent in the seeds.

Keywords: Lectin, Hemagglutinating activity, *Pterocarpus osun*, antioxidant, agglutination.

1. INTRODUCTION

Lectins are sugar-binding proteins or glycoproteins which agglutinate erythrocytes and are widely distributed in nature. Lectins have been isolated from various biological sources such as plants, animals and micro-organisms [1,2]. Lectins have been a subject of intense study for more than a decade because they possess various biological activities such as mitogenic and antiproliferative, antiinflammatory, antitumor, antifungal, antibacterial, vasorelaxant, antioxidant

and antihemolytic among others [2-8]. Lectins have the ability to recognize carbohydrate or glycoconjugate and reversibly bind to it through its carbohydrate-binding sites. The binding is with high affinity and specificity and without any chemical modification because lectin has no enzyme-catalytic activity. Lectin can also agglutinate other cells apart from red blood cells. These distinguishes lectin from other carbohydrate-binding proteins and make them valuable tools in biotechnological, pharmacological and therapeutic applications [5,9].

The richest sources of lectins in plants are mature seeds, especially those of the legumes, where lectins may constitute one tenth of the seed total protein. Legume lectins are model system of choice to study the molecular basis of protein-carbohydrate interactions because they are not only easy to purify in large quantities, but also exhibit a wide variety of carbohydrate specificities despite strong sequence conservation [10]. The large majority of the leguminous lectins that have been isolated and characterized are from plants belonging to the tribe of *Phaseoleae* and *Dalbergieae* of the *Papilionoideae* subfamily of leguminosae [11].Worthy of mention is *Pterocarpus angolensis* seeds lectin which has been purified and physicochemically, biochemically and structurally characterized [12,13].Other seed lectins that have been purified and biochemically characterized from this tribe include galactose-binding lectins from *Lonchocarpus capassa* [14], *Vatairea macrocarpa* [11]and *Vatairea guianensis* [15] and mannose-binding lectins from *Platymiscium floribundum* [16] and *Centrolobium tomentosum* [17]. Amino acid sequence of *P. floribundum* [16], *C. tomentosum* [17] and *Centrolobium microchate* [18] lectins has been determined partially. Generally, legume lectins are structurally homologous and at time have similar physicochemical properties but display biological activity that are distinctly differ. Consequently, each of the lectins has the potential for different application and deserves to be independently studied.

Pterocarpus osun Craib belongs to the *Dalbergieae* tribe of Papilionaceae subfamily. *P. osun* is endemic to Southern Nigeria, Equatorial Guinea, Gabon, Cameroun and Zaire [19]. It exists as a tree of about 30 meters height and 2.5 meters girth with a spreading crown and the wood marketed as African Padauk. The leaves of *P. osun* are used in the treatment of skin disease such as eczema, candidiasis and acnes [20]. The crude extract of *P. osun* has also been found useful in the treatment of chicken pox in children in the eastern part of Nigeria [21]. The antioxidant potential and the attenuation of acetaminophen-induced redox imbalance by *P. osun* were reported recently [22]. Adewuyi *et al.* [23] showed that the acetonides prepared from the seed oil of *P. osun* has no antibacterial activities but the leaves ethanolic extract of the plant does.

In our preliminary study [24], the presence of hemagglutinin in the crude protein extract of *P. osun* seeds was established but the lectin was not purified and characterized. The present study, therefore, focused on purification and

physicochemical characterization of lectin from *P. osun* seeds and also, we investigated its *in-vitro* antioxidant and antibacterial activities.

2. MATERIALS AND METHODS

2.1 Preparation of crude extract

The dried mature seeds of *Pterocarpus osun* were removed from the pods and ground into powder using seed blender, after which 50 g of the powder was defatted using petroleum ether and later was extracted in ten volumes of 25 mM phosphate buffer saline (PBS, pH 7.2) containing 10mM sodium chloride. After stirring on magnetic stirrer for about 10 hrs, the mixture was centrifuged at 10,000 rpm for 20 min and the supernatant was collected into a sample bottle and stored at – 20 ^{o}C until used.

2.2 Erythrocytes glutaraldehyde fixation and trypsinization

Glutaraldehyde was used to fixed the erythrocytes of human and animal bloods following the methods described by Kuku and Eretan, [25]. Heparinized bottles were used to collect the blood samples, which was centrifuged at 3,000 rpm for 15 min. The erythrocytes were thoroughly washed with PBS, pH 7.2. 2% of the erythrocyte was prepared with chilled 1% glutaraldehyde-PBS (v/v) solution. The suspension was incubated at 4 $^{\circ}$C for 1 hr with occasional mixing. This was followed by centrifugation at 3,000 rpm for 5 min and several washing of the fixed blood cells with PBS to remove glutaraldehyde. The fixed cells were suspended in PBS to a final concentration of 2%. Trypsinization of the erythrocytes was carried out as described by Occena *et al*. [26]. 2% erythrocytes suspension in PBS was obtained by thoroughly washing the whole blood samples of blood groups A, B, O and animals with PBS. Equal volume of 2% erythrocytes suspension and 1% trypsin solution was mixed an incubated for 1 hr at 37 $^{\circ}$C. The trypsinized cells were washed five times with PBS and finally diluted to obtained 2% (v/v) trypsinized cells in PBS. This was stored until further use.

2.3 Hemagglutination assay and blood group specificity

A two-fold serial dilution of *P. osun* seeds lectin solution (100 µl) was performed in U-shaped microtitre plates. This was mixed with 50 µl of a 2% suspension of human as well as animal (rabbit and rat) or 2% trypsinized erythrocytes in phosphate buffered saline, pH 7.2 at room temperature. The erythrocytes have been preciously fixed with 1% glutaraldehyde. The plate was left undisturbed for 2 hr for agglutination to take place. The hemagglutination titre of the lectin expressed as the reciprocal of the highest dilution of the lectin exhibiting visible agglutination of erythrocytes was

equivalent to one hemagglutinating unit. Specific activity was the number of hemagglutination units per mg protein. The blood group specificity of the crude lectin extract was determined using erythrocytes from different blood groups of the ABO system and those of the rabbit and rat.

2.4 Sugar specificity test

The sugar specificity of the lectin was investigated by the ability of sugars to inhibit the agglutination of human erythrocytes [27]. A serial dilution of the crude lectin sample was made until the end-point causing hemagglutination was obtained. 0.2 M of each sugar solution was added to each well at 50 µl per well and allowed to stand for 1 hr undisturbed on the laboratory bench and then mixed with 50 µl of 2% rabbit erythrocyte suspension. The hemagglutination titre obtained were compared with a non-sugar containing blank. The sugars tested are: maltose, D (+)-mannose, lactose, L (+)-arabinose, sorbose, D (+)-glucose, sucrose, galactose, mannitol, N-acetyl-D-glucosamine, mannosamine, 2-deoxy-D-glucose, dulcitol, xylose, α-D-methyl glucopyranoside and D (+)-glucosamine HCl, α-D-methyl-mannoside.

2.5 Temperature, pH and EDTA Effect on hemagglutinating activity

Thermal stability of the lectin was tested by incubating the purified lectin at different temperature ranging from 30 $^{\circ}$C – 100 $^{\circ}$C in a water bath for 1 hr. At 15 min interval, for each temperature, hemagglutinating activity of aliquots taken was determined by hemagglutination assay. Control was the lectin kept at room temperature and represent 100% hemagglutinating activity.

Hemagglutinating activity of the lectin at both basic and acidic condition was tested. The purified lectin was incubated with buffers of different pH values ranging from pH 3.0 – 13.0. for 1 hr. hemagglutinating activity of the lectin was determined and compared with the control which was lectin incubated in PBS (pH 7.2). Buffers used were 0.2 M citrate buffer (pH 3.0 - 5.0), 0.2 M Tris-HCl buffer (pH 6.0 - 8.0) and 0.2 M glycine-NaOH buffer (pH 9.0 - 13.0).

To determine if the lectin require divalent metal ion for its hemagglutinating activity, lectin was dialyzed against 10 mM and 100 mM EDTA for 24 hrs. Hemagglutinating activity of the resulting lectin was determined. This was followed by incubating the treated lectin with 10 mM of each of the following divalent cation salts: $CaCl_2$, $MgCl_2$, $MnCl_2$, $BaCl_2$ and $SnCl_2$ for 2 hrs in order to determine if the hemagglutinating activity be restored.

2.6 Purification of P. osun lectin

2.6.1 Ammonium sulphate precipitation

The crude lectin extract of the *P. osun* seeds was subjected to 70% ammonium sulphate precipitation. The ammonium sulphate equivalent to 70% precipitation was slowly and continuously added to the crude extracts on magnetic stirrer to aid dissolution of the salt. The mixture was centrifuged after 24 hrs at 3500 rpm for 15 min to obtain the precipitate. The precipitate was dialyzed exhaustively against several changes of PBS to remove the salt. The dialysate was centrifuged at low speed to remove undissolved materials.

2.6.2 Gel-filtration on Sephadex G-100

The dialysate of ammonium sulphate precipitate of *P. osun* crude lectin extract was applied on Sephadex G-100 column (2.5 x 40 cm) previously equilibrated with PBS, pH 7.2. The protein was eluted with the same buffer at a flow rate of 15 ml/hr and 5 ml fractions were collected. The fractions were monitored for protein by measuring the absorbance at 280 nm and assayed for hemagglutinating activity.

2.6.3 Determination of protein concentration

Protein concentration of the crude extract, dialysate and other fractions were determined by the method of Lowry *et al.* [28] using Bovine Serum Albumin (BSA) as standard protein. The absorbance at 280 nm was also used to monitor protein elution in the chromatographic fractions.

2.7 Physicochemical characterization of purified lectin

2.7.1 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

Ability of the lectin to scavenge DPPH radical was evaluated by method described by Brand-Williams *et al.*[28] with slight modification. Equal volume (1 ml each) of 0.3 mM DPPH and varying concentration of lectin or standard (ascorbic acid) were mixed. The mixture was incubated in the dark for 30 min. Negative control was prepared by addition of 1 ml methanol instead of lectin. Absorbance of the test and control was read at 517 nm. The percentage of DPPH radical scavenging activity inhibition was obtained using this equation.

$$\text{DPPH radical scavenging inhibition \%} = \left[1 - \left(\frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\right)\right] \times 100$$

Where:

$\text{Abs}_{\text{sample}}$ = Absorbance of the lectins

$\text{Abs}_{\text{control}}$ = Absorbance of the control at 517 nm

Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph by plotting inhibition percentage against sample concentration.

2.7.2 Lipid peroxidation inhibition assay

Lipid peroxidation was carried out according to the methods of Ohkawa *et al.* [30] as described by Hattori *et al.* [31] with slight modification and BHT was used as standard. 10% egg yolk homogenate was prepared in 150 mM Tris-HCl buffer (pH 7.2). Five hundred microlitres (500 µl) of the egg yolk homogenate was added to 0.1 ml of varying concentration of the purified lectin and standard (BHT) separately. Then, 50 µl of 1% ascorbic acid was added to the reaction mixture, followed by 50 µl of 0.07 M $FeSO_4$ to induce lipid peroxidation. The reaction mixture was vortexed and incubated at 37 $^{\circ}$C for 1h. Sequential addition of 0.5 ml of 0.1N HCl and 2 ml of 0.67% (w/v) Thiobarbituric acid prepared in 9.8% SDS followed incubation. The resulting mixtures were heated in water bath at 95 $^{\circ}$C for 1 h, cooled and 2.0 ml of butan-2-ol was added and later centrifuged at 3,000 rpm for 10 min. The control was run as above with the lectin/standard replaced with distilled water. The supernatant was collected and measured at 532 nm. Percentage inhibition of lipid peroxidation was calculated as:

$$\% \text{ Inhibition} = \frac{Abs_{control} - Abs_{test}}{Abs_{control}} \times 100$$

where $Abs_{control}$ = MDA produced by fenton reaction in the absence of extract (control);

Abs_{test}= MDA produced by fenton reaction in the presence of extract.

2.7.3 Ferric reducing antioxidant power (FRAP) assay

Ferric reducing antioxidant power assay was carried out spectrophotometrically adopting the method described by Benzie and Strain [32] with minor modification. The FRAP working reagents was prepared by mixing ten parts of 300 mM acetate buffer (pH 3.6), one part of 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) and one part of 20 mM of ferric chloride solution in the dark. Fifty microlitres of varying concentration of the lectin and standard solution of the ascorbic acid was added to 1 ml of FRAP working reagent. The mixture was vortexed before incubating at 37 $^{\circ}$C for 30 min in the dark. The absorbance was taken at 593 nm against the reagent blank containing 1 ml of the FRAP working reagent and 50 µl of methanol. All measurements were taken at room temperature and the reducing power was expressed as equivalent concentration which is defined as the concentration of antioxidant that gave a ferric reducing ability equivalent to that of the ascorbic acid standard (AAE).

2.8 Antibacterial assay

2.8.1 Antibacterial sensitivity test

The *in vitro* sensitivity tests of the bacteria to the purified lectins were done by disc diffusion method described by Akinpelu *et al.* [33] with little modification. About 1 ml of the standardized 24 hrs old culture of the test organisms in

nutrient broth was inoculated into pre-sterilized molten Mueller-Hinton agar medium in MacCartney bottle. The medium was poured into a sterile Petri dish and allowed to set. With the aid of a sterile cork borer, three wells of about 6 mm in diameter were bored on the plates equidistant from the centre of the plates. About 0.1 ml of each of the purified lectins (5 mg/ml) was dispensed into the wells in each of the Petri dishes. The same volume of antimicrobial standard drugs-streptomycin (1 mg/ml) was dispensed into the third well in the Petri dishes. The plates were incubated at 37 °C overnight. At the end of the incubation period, zones of inhibition formed on the agar plates were measured. Zones of inhibition indicate susceptibility of the test bacteria to the lectin suspension and were evaluated in mm.

2.8.2 Bacterial agglutination test

Bacteria were tested for agglutination with the purified lectins. Both Gram negative and Gram-positive bacteria were grown in nutrient broth for about 24 hrs. The cells were harvested by centrifugation at 3000 rpm for 2 minutes and washed with PBS three times. The packed cells were suspended in 0.5% formalin solution and shaken at 25 °C for 24 hrs. Formalin-killed cells were collected by centrifugation, washed with PBS and resuspended in PBS to 1.5×10^{8} colony forming unit/ml (McFarland 0.5 standard). Agglutination assay with the formalin-killed cells was performed in microtitre plates. An equal volume of each bacterial suspension was mixed with a two-fold serial dilution of the lectin in a microtitre plate and incubated at room temperature for one hour. The bacterial agglutination titre was expressed as the reciprocal of the highest dilution giving a visible agglutination upon illumination of the microtitre plates [34].

3. RESULTS AND DISCUSSION

Pterocarpus osun seeds lectin (POSL) was easily purified by combination of salt precipitation using ammonium sulphate and size exclusion chromatography on Sephadex G-100. The soluble crude protein extract obtained by PBS extraction of *P. osun* seeds powder was initially precipitated by addition of ammonium sulphate up to 70% saturation and active dialysate obtained after exhaustive dialysis was layered on Sephadex G-100 gel filtration column. The elution profile (Figure 1) presented three distinct protein peaks (GO1, GO2, GO3), where only the third peak (GO3) displayed hemagglutinating activity against rabbit erythrocyte. Similar purification procedures were employed by Galbraith and Goldstein [35] and eLacerda *et al*. [36]. In both studies, ammonium sulphate precipitation of the protein preceded size exclusion chromatography on Sephadex G-200 and Sephadex G-100, respectively. Three distinct protein peaks were obtained by eLacerda *et al*. [36] who worked on Brazilian lima bean variety and only the first peak exhibited hemagglutinating activity. The specific activity of the purified lectin was 119.1 HU/mg protein leading to protein purification of 46-fold (Table 1).

Phosphate buffer saline extraction produced a soluble crude lectin extract that showed measurable hemagglutinating activity against trypsin-treated and native rabbit erythrocyte with higher hemagglutinating titre for enzyme-treated erythrocyte. The crude lectin extract was unable to agglutinate native human erythrocyte but trypsinized-human erythrocyte of all ABO blood group was considerably and non-specifically agglutinated. The results are shown in Table 2. Similar results were reported for lectins from *Platymiscium floribundum* [16], *Centrolobium microchaete* [18] and *Canavalia virosa* [37]. Lis and Sharon [38] revealed that trypsin can be used to modify the erythrocytes surface to enhance its affinity for lectins without affecting the total number of lectin binding sites on the erythrocytes. In supporting this statement Singh and Saxena [39] stated that trypsinization of red blood cells may removes the sialoglyocpeptide of the cells; thus, demolishing the negative charge on the surface of the cells, which may lead to decrease in repulsive force between the cells and hence increase in agglutination.

Inhibition of hemagglutination by several different sugars showed that the lectin activity was strongly inhibited by glucose, its epimer – mannose and their derivatives like, 2-deoxy-D-glucose, N-acetyl-D-glucosamine, α-D-methyl-mannoside, α-D-methyl-gluocpyranoside and a disaccharide (maltose). Complete inhibition of the hemagglutinating activity was noticed with mannose, α-D-methyl-mannoside and maltose. Maltose exhibited most potent inhibitory effect with minimum inhibitory concentration of 260μM followed by α-D-methyl-mannoside and mannose (Table 3). These results indicate that presence of another glucose unit at the carbon-1 of the first glucose in the disaccharide increases the interaction with the hydrophobic regions of the carbohydrate-binding site, thereby increased the affinity of the POSL when compared with glucose. Availability of methyl group on α-methyl-mannoside may also causes the same interaction that resulted in higher affinity of the POSL for α-methyl-mannoside than mannose. POSL belongs to the mannose/glucose specificity group of lectins from Dalbergieae tribe, which have specificity for different sugars. Among well studied member of the tribe that belong to mannose/glucose specificity group are lectins from *Pterocarpus angolensis* [3], *Platymiscium floribundum* [16], *Centrolobium microchaete* [18], and *Platypodium elegans* [40]. Though, other members of the tribe that have specificity for other carbohydrates especially galactose have also been reported (*Vatairea marcocarpa* [11]; *Lonchocarpus capassa* [14]; *Vatairea guianensis* [15]).The biological importance of mannose-binding lectin also has been stretched [41].

POSL was thermostable, demonstrating full activity up to 70 $^{\circ}$C during 15 min of heating. Fifty percent of the full activity was lost when heated for 60 min at 70 $^{\circ}$C while retaining 100% activity at 60 $^{\circ}$Cfor 60 min and no hemagglutinating activity was detected when the lectin was heated at 80 $^{\circ}$C for 15 minutes (Figure 2 A and B). This implies that the lectins undergo conformational changes under extreme temperatures resulting in the loss of activity. The loss of activity of the lectins with increased temperature is due to destabilization of sporadic weak interactions of tertiary structure responsible

for native conformation of lectin [39]. These results are comparable to the reported results of lectins purified from *Vatairea marcocarpa* [11], *Vatairea guianensis* [15], *Platymiscium floribundum* [16], and *Canavalia oxyphylla* [42]. In contrast, extremely thermostable lectins have been reported from *Bauhinia forficate* [43] and *Apuleia leiocarpa* [44]. These lectins retain maximum hemagglutinating activity when heated at 100 °C. High thermostability possessed by these lectins may be advantageous, as stable bioactive substance is more efficient during all phases of their processing and on the other hand this is considered as antinutritional factors that cause many adverse phenomena in animals if ingested [36].

Lectin, generally, are found stable in harsh conditions such as extreme pH. POSL was subjected to hemagglutination assay at different pH and the lectin retained maximum activity within a broad pH range (pH 3-13) (Figure 3). The result suggest that the lectin was insensitive to acidic and basic pH. *Phaseolus lunatus* seeds lectin exhibited hemagglutinating activity within a broad range, remaining stable between pH 2 and 11 [36]. Other lectins with similar pH optimum have been reported [45,46]. Some lectins have shown that extreme pH is less favorable conditions for their hemagglutinating activity. They are found to display maximum activity at around neutral pH. Lectins from *Vatairea guianensis* [15], *Platymiscium floribundum* [16] and *Centrolobium microchaete* [18] retained full hemagglutinating activity at pH 6-9. They all belong to the same Dalbergieae tribe with the *P. osun*.

The hemagglutinating activity of POSL remain unchanged after dialysis against or incubation with high concentration of EDTA and addition of divalent cations to the EDTA-treated lectin also did not alter the activity. These probably suggest that POSL does not need divalent cations for it to be active or the metal ions are tightly bound to the lectin. The hemagglutinating activity of lectin from *Vatairea guianensis* [15] and *Platymiscium floribundum* [16] among others showed similar results when incubated with EDTA. Although, this is in contrast to the *P. angolensis* [13] and *Centrolobium microchaete* [18] lectins that completely lost their activity after treatment with EDTA and only addition of metal ions restored their full lectin activity.

Apart from peptides, obtainable by either enzymatic hydrolysis, chemical hydrolysis or bacterial fermentation, that have been established to possess strong antioxidant ability, numerous evidences exist that proteins possess antioxidant activity and that these antioxidant proteins have been closely linked to the control of some neurodegenerative and cardiovascular diseases because of its ability to ameliorate the harmful effect of free radicals and reactive oxygen species produced during oxidative stress. DPPH radical scavenging, lipid peroxidation inhibition and ferric reducing antioxidant power assays were used to assess the antioxidant potential of POSL. The results revealed that POSL possess significant antioxidant activity, which were concentration dependent (Figure 4A and B). The lectin showed an IC_{50} of 1.17 ± 0.08, 0.58 ± 0.03, and 2.51 ± 0.03 mg/ml for those methods respectively. These results give support to reported studies that

detected lectins with antioxidant potential in some leguminous seeds [35, 46-47] and also to other reported antioxidant proteins from other plant family [48-50]. Though, antioxidant activity in protein possibly will not be ascribed to a single mechanism. Elias *et al*. [51] stated some plant proteins can inhibit lipid oxidation via numerous pathways and inactivate reactive oxygen species and other free radicals, chelate transition metals and reduce hydroperoxides. Presence of some amino acids in the primary structure of this lectin may have contributed to the observed antioxidant ability. Therefore, hydrolysis of POSL may ascribe more antioxidant potential to the peptides that will be generated.

The antimicrobial roles of lectins as stated by Coelho *et al*. [52] include blockade of invasion and infection, inhibition of growth and germination, regulation of microbial cell adhesion and migration. There is an increasing interest in investigation of the lectin's involvement in the interaction between eukaryotic cells and pathogens in infectious disease development and their antimicrobial potential [53]. Carvalho *et al*. [43] reported that *Apuleia leiocarpa* seed lectin (ApulSL) demonstrated bacteriostatic effects on the Gram-positive bacteria *Staphylococcus aureus, Streptococcus pyogenes, Enterococcus faecalis, Micrococcus luteus, Bacillus subtilis* and *Bacillus cereus*, and on the Gram-negative bacteria *Xanthomonas campestris, Klebsiella pneumoniae, Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enteritidis*. ApulSL was also bactericidal against three varieties of *Anthomonas campestris*. Also, in their studies Mishra *et al*. [54] showed that *Bauhinia variegata* lectin (BVL) demonstrated a remarkable antibacterial activity against the pathogenic bacteria *P. aeruginosa*, *S. aureus*, *E. coli*, *and B. subtilis*. BVL also shows a significant antifungal activity against *Aspergilus niger* and *Penicilium crysogenum.* The results of the present antibacterial study are contrary to the majority of the lectins from leguminous family. The present study showed that purified POSL have no antibacterial activity against both gram-positive and gram-negative bacteria strain used and was unable to agglutinate these pathogens. But the crude protein extract demonstrated significant antibacterial effect against gram-positive bacteria (*B. cereus, S. aureus* and *B. subtilis*) and gram-negative bacteria (*Pseudomonas fluorescens*, *K. pneumoniae*, *E. coli, P. aeruginosa* and *Proteus vulgaris*). But also, could not agglutinate them. I can therefore be concluded that the antibacterial activity exhibited by the crude protein extract is not due to the presence of lectin but possibly to another antibacterial proteins or peptides. Several antibacterial peptides and proteins have been isolated from plants [55,56].

4. CONCLUSION

Pterocapus osun seeds lectin (POSL) was purified and characterized. POSL was in inhibited by mannose, maltose and α-methylmannoside, indicating that it belongs to mannose/glucose-binding lectin. POSL agglutinated native rabbit erythrocytes and nonspecifically enzyme-treated human blood group ABO erythrocytes. The lectin was found stable up to

70 ^{o}C, active in a wide pH range and requiring no divalent cations for its full activity. No antibacterial activity was detected but exhibited significant antioxidant activity.

CONFLICT OF INTEREST

There are no conflicts of interest among the authors.

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Table 1: Summary of purification procedure for *Pterocarpus osun* seeds lectin (POSL)

Fractions	Volume (ml)	Total protein (mg)	Total Activity (HU)	Specific Activity (HU/mg)	Fold Purification
Crude protein extract	50	394.0	1024	2.6	1.0
70% Ammonium Sulphate Precipitate Dialysate	18	77.4	1024	13.23	5.1
Gel Filtration Sephadex G-100	6	12.9	2048	158.8	61.1

Table 2: Hemagglutinating activity of PBS extract of *P. osun* against human and animal erythrocytes

Erythrocyte		Non-trypsinized	Trypsinized
Human	A	2^0	2^5
	B	2^0	2^5
	O	2^0	2^4
Rabbit		2^{11}	2^{15}
Rat		2^6	ND

ND Not determined.

Table 3: Inhibition of hemagglutinating activity of *P. osun* seed lectin by different sugars.

Sugars	Hemagglutinating Titre	Minimum Inhibition Concentration (mM)
Arabinose	2^{11}	ND
Xylose	2^{9}	ND
Glucose	2^{3}	0.913 ± 0.345
Galactose	2^{9}	ND
Mannose	**2^{0}**	1.824 ± 0.689
Sorbose	2^{10}	ND
Maltose	**2^{0}**	0.260 ± 0.065
Sucrose	2^{5}	ND
Lactose	2^{11}	ND
Mannosamine	2^{7}	ND
Glucosamine HCl	2^{5}	ND
2-deoxy-D-glucose	2^{2}	3.646 ± 1.378
N-acetyl-D-glucosamine	2^{3}	ND
α-D-methyl glucopyranoside	2^{2}	1.043 ± 0.261
Mannitol	2^{11}	ND
Dulcitol	2^{9}	ND
α-methyl mannoside	**2^{0}**	0.456 ± 0.173
Control	2^{11}	ND

Minimum inhibition concentration is the minimum concentration of sugar that inhibits 50% of hemagglutinating activity. Data for minimum inhibition concentration are expressed as mean ± SEM of triplicate determination
ND - Not determined

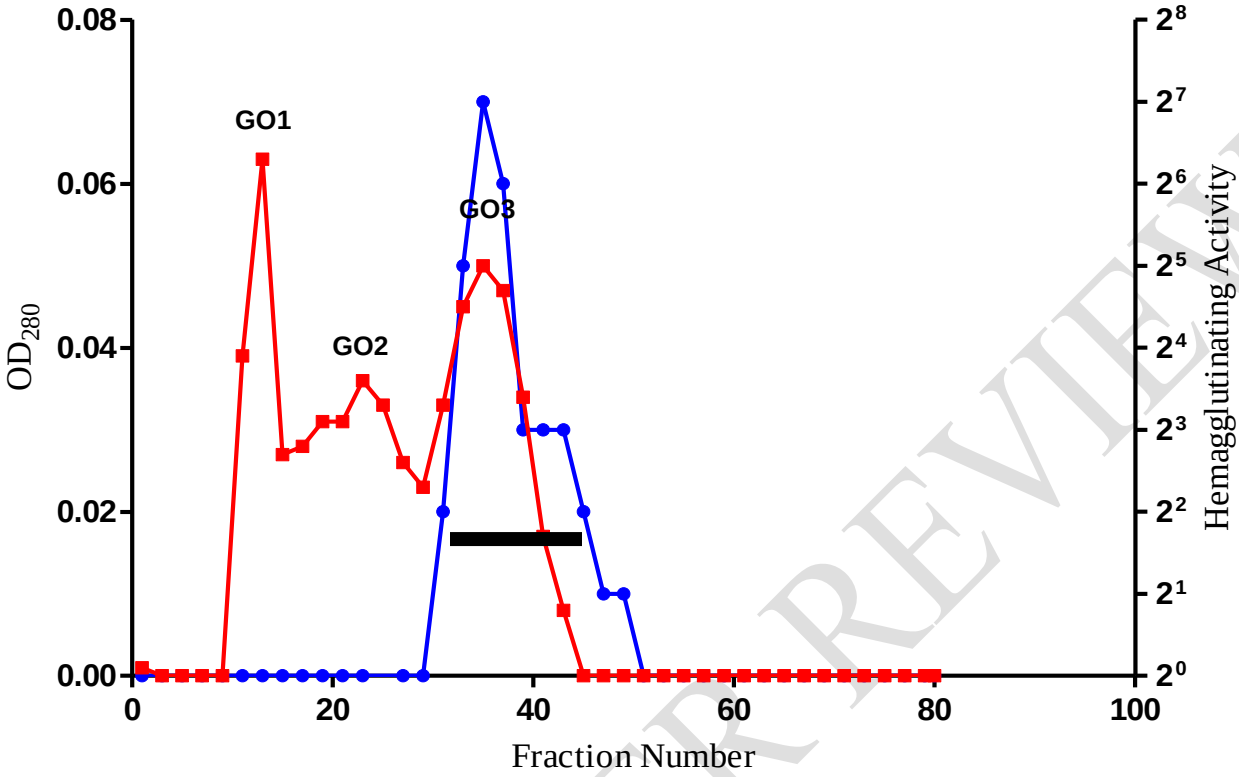


Figure 1: Gel filtration chromatogram of ammonium sulphate dialysate of crude extract of *P. osun*seeds on Sephadex G-100 column.

The column (2.5 x 40 cm) packed with Sephadex G-100 was equilibrated with 25mM phosphate buffered saline (PBS) pH 7.2 containing 10mM sodium chloride (NaCl). 5 ml of ammonium sulphate precipitate dialysate (4.3 mg) was layered on the column and the lectin was eluted with the same buffer at a flow rate of 15 ml/hr and fractions of 5 ml were collected.

Legend: ━━ Pooled fractions; ••••• Hemagglutinating activity; ■■■■ OD_{280}; GO - Protein Peaks

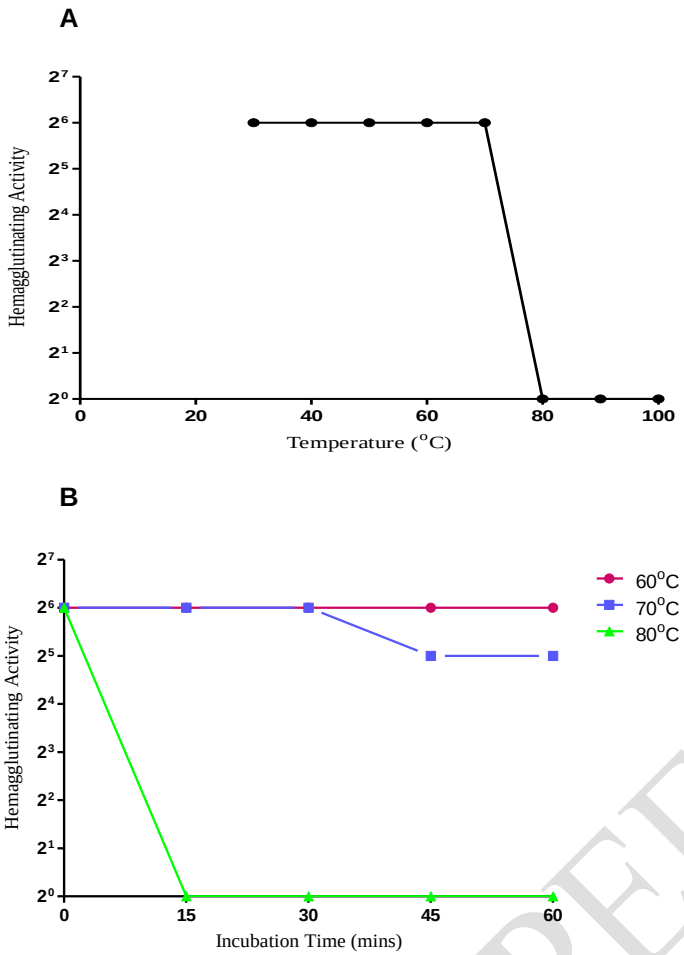


Figure 2: (**A**) Effect of temperature on *P. osun*seeds lectin (**B**) Thermostability of *P. osun*seeds lectin. Lectin samples were incubated at different temperatures (30 - 90°C) for 60 min. Aliquots of the lectin was taken at every 15 min interval and then rapidly cooled in ice and assayed for agglutinating activity. The control was agglutinating activity of lectin sample kept at 20°C.

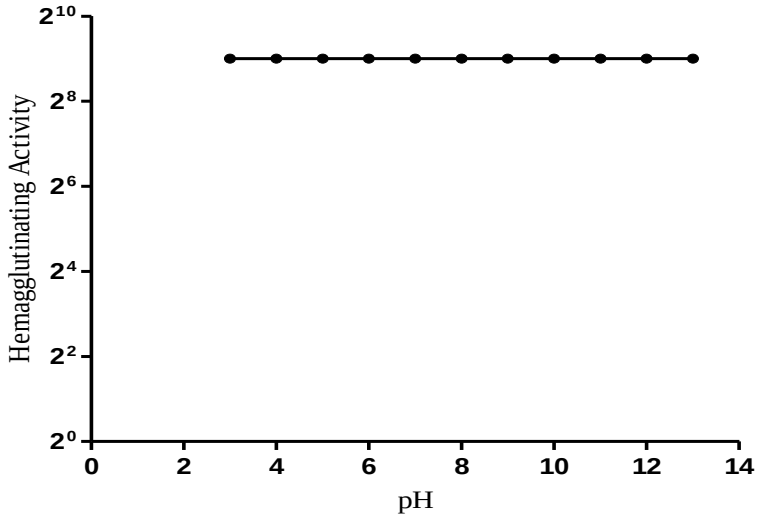
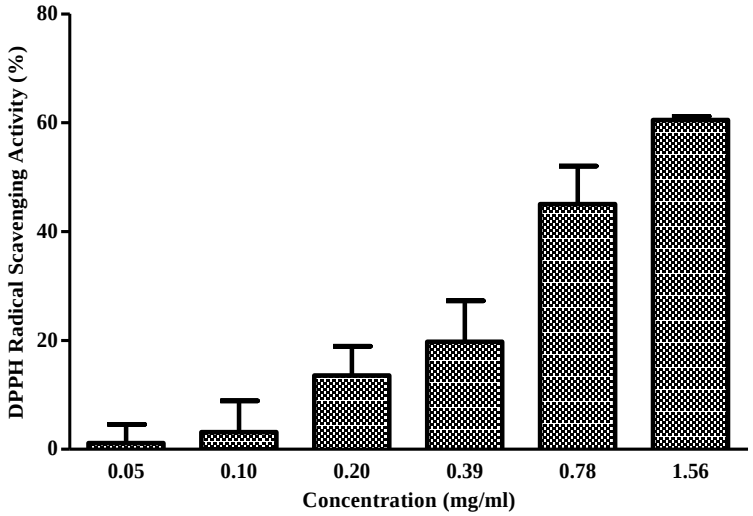


Figure 3: Effect of pH on the hemagglutinating activity of *P. osun*seeds lectin.

Lectin samples were incubated in the following buffers at different pH values; 0.2 M citrate buffer, pH 2.0 – 5.0; 0.2 M Tris-HCl buffer, pH 6.0 – 8.0; and 0.2 M glycine-NaOH buffer, pH 9.0 – 13.0. After 1 hour, the hemagglutination activity of the lectin was determined. The control values were the agglutination titre of the lectin in PBS, pH 7.2.

A



B

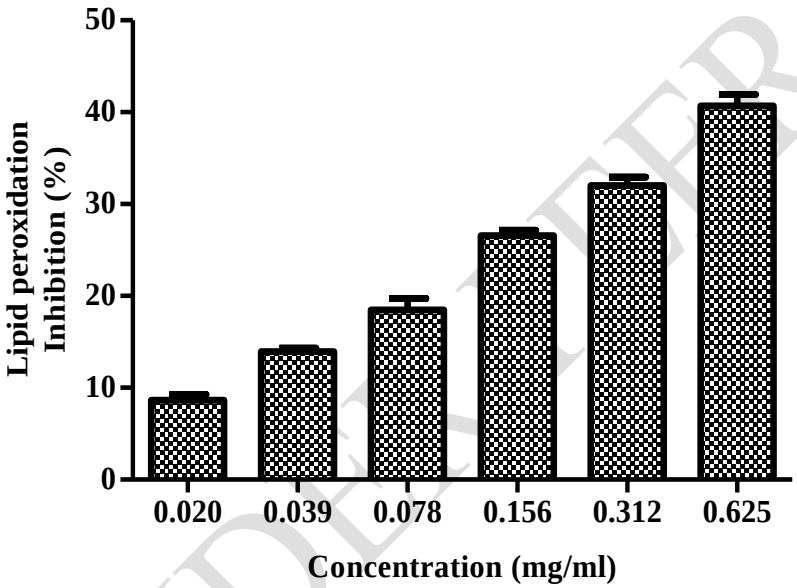


Figure 4: Antioxidant activity of POSL (A) DPPH radical scavenging activity of *P. osun*seeds lectin (B) Lipid peroxidation inhibition. Data are expressed as mean ± SEM. (n=3)